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"Further Note on some Additional Points in Connection with Chloroformed Calf Vaccine."

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(From the Government Lymph Laboratories.)

Since April, 1903, the date of my preliminary note* on this subject, the preparation of calf vaccine by the chloroform process has been carried on in at these laboratories and a large number of vaccines have now been treated by this method. These lymphs have been freed from their non-sporebearing extraneous bacteria within a period ranging between 1 and 8 hours after their collection from the calf, and have, subject to the usual tests, been issued for general vaccination purposes about 2 weeks after collection. Their use has resulted in high "case" and "insertion" success.

The following points in connection with these vaccines have been investigated.

The Effect of Temperature in the Elimination of Extraneous Micro-organisms from Crude Calf Vaccine by the Chloroform Process.

It has been ascertained that the temperature at which vaccine emulsion is subjected to the chloroform process determines largely the rate at which the extraneous bacteria of that emulsion are eliminated. This has been shown in two ways.

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* 'Roy. Soc. Proc.,' 1903.

In the first set of experiments vaccine emulsion, consisting of one part by weight of pulp and two parts by weight of water, was divided into several equal portions. Each portion was then submitted to a pre-determined and separate temperature along with the set of apparatus to be used for its treatment with chloroform. These temperatures ranged from 10° C.—37° C. In each case after both emulsion and apparatus had reached the requisite temperature of the experiment, passage of chloroform vapour and air through the emulsion was effected.

It was found by means of plate cultivations, that elimination of extraneous micro-organisms was most rapidly effected in the case of the emulsions chloroformed at 37° C., and that elimination was most slowly effected in the case of the emulsions chloroformed at 10° C. Between these temperatures the gradation of germicidal action was very constant.

The potency of these vaccines was subsequently tested by inoculations on calves, with the result that vaccines which had been subjected to the chloroform process at temperatures below 30° C. caused rather better vesiculation than the vaccines treated at temperatures above 30° C.

In the second set of experiments, vaccine emulsion, prepared as before, was divided into a number of equal portions, each of which was placed in a separate test-tube. In this case, the emulsions were all subjected to the chloroform process at 15° C. After the passage of chloroform vapour and air through the emulsions for half an hour, the entrance and exit of each vaccine tube were clamped in order that none of the contained chloroform might escape, and the tubes were placed in temperatures ranging from 10° C—37° C. for 24 hours In the case of these emulsions, where the amount of chloroform present in each must have been approximately the same, a similar gradation of germicidal action was evidenced as in the former set of experiments; elimination occurring most rapidly in the case of emulsions submitted to a temperature of 37° C., and most slowly in the case of emulsions submitted to a temperature of 10° C.

The potency of each of these vaccine emulsions was tested as before by inoculations on calves, with the result that vaccines which had been submitted to temperatures below 30° C. gave slightly better vesicles than vaccines which had been submitted to temperatures above 30° C.

Both series of experiments indicate that the temperature at which extraneous bacteria are killed most quickly in vaccine emulsion by the chloroform process, the specific germ being left meanwhile in state of full activity, lies probably between 18° C. and 23° C.

The Species of Micro-organisms Killed by the Chloroform Process.

In addition to the extraneous micro-organisms mentioned* as commonly occurring in crude calf vaccine and as being eliminated therefrom by means of chloroform, experiments have been made with further species of bacteria. These, with the exception of *B. proteus vulgaris* and *B. coli communis*, have never been found in vaccine lymph at these laboratories, but they have been subjected to the action of chloroform in order that knowledge of the germicidal value of that process might be extended. The bacteria thus experimented with are:—*B. proteus vulgaris*, *B. prodigiosus*, *B. pyocyaneus*, *B. fluorescens liquefaciens*, *B. coli communis*, *B. typhosus*, *B. diphtheriæ*, *B. mallei*, *B. pestis*, *B. tuberculosis*, *and Spirillum choleræ Asiaticæ.*

Broth emulsions of these bacteria were first experimented with. As soon as an emulsion was established a suitable culture medium was inoculated with a small portion of it and duly incubated, in order to ascertain whether the species of micro-organism in question could be easily recovered from the emulsion. The passage of chloroform vapour and air through the emulsion was then begun, and during the process at regular intervals further cultivations were made from it. In every instance the bacteria of experiment were found to have been killed, at intervals ranging from 1—8 hours from the commencement of the process.

In a second set of experiments vaccine emulsions were prepared by mixing one part by weight of pulp with two parts by weight of water. To these emulsions the bacteria to be experimented with were added, and cultivations at once made therefrom in each instance, to ascertain whether recovery of the bacteria was possible. Passage of chloroform vapour and air through the emulsions was then carried out and further cultivations made from them from time to time. As in the case of the broth emulsions, after the passage of the chloroform vapour and air for a few hours, the bacteria in the vaccine emulsions were killed.

In the case of each experiment of the two foregoing series, a "control" preparation was established in which the bacteria were found to be still alive after the bacteria of the corresponding experimental preparation had been killed.

The vaccine used in these experiments was collected for experimental purposes only.

The Keeping Properties of Chloroformed Vaccines.

The keeping properties of chloroformed vaccines have been tested in the following ways:

First, vaccine lymph freshly collected from the calf was divided into two portions. One portion after emulsification with water was subjected to the chloroform process and, after elimination of its extraneous micro-organisms, the chloroform was partly removed by the passage through the emulsion of a stream of sterile air. The other portion was emulsified with 50 per cent. of glycerine and water solution. These chloroformed and glycerinated emulsions were, within 24 hours of their collection from the calf, each sub-divided into five parts, one part of each being stored at 10° C., 20° C., 25° C., 30° C., and 37° C. The potency of these several parts was subsequently tested on calves at regular intervals of time. The result showed: (*a*) that the highest potency was retained for the longest time, alike for the chloroformed and glycerinated portions, by the emulsions stored at 10° C., and for the shortest time, alike again by both portions, by emulsions stored at 37° C.; and (*b*) that the potency as between the chloroformed and glycerinated vaccine kept at any one of the foregoing temperatures was practically the same, the only marked difference occurring in case of the vaccines kept at 37° C., a temperature at which the chloroformed emulsions retained potency for a longer period than did the glycerinated emulsions.

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* 'Roy. Soc. Proc.,' 1903.

Upon the completion of the foregoing experiments, a further number of vaccines collected from vesicles of good average quality were emulsified with water in the proportion of one part by weight of pulp to two parts by weight of water, and subjected as before to the chloroform process. When elimination of their extraneous bacteria had been effected, part of the chloroform was removed from the emulsions and these were placed in the ice chest at 10° C. Immediately prior to the issue of these vaccines, the remainder of the chloroform was removed and glycerine added in the proportion of two parts by weight of glycerine to the original weight of vaccine pulp. These vaccines were later, after passing the requisite tests, issued for general vaccinating purposes, a similar interval being allowed to elapse between their collection from the calf and subsequent issue as is usual at these laboratories in the case of glycerinated vaccine, namely, about 6 weeks. In the case of two of these chloroformed vaccines an interval of 2 months elapsed between collection and issue.

The use of these chloroformed vaccines has been attended with results showing high "case" and "insertion" success.

Summary.

Experience of the further use of the chloroform process in the preparation of a large number of vaccines during the past year confirms the conclusions arrived at in a former paper.* And mean-

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* *Loc. cit.*, 1903.

while important additional knowledge has been gained, namely, that chloroformed calf vaccine, if originally of sufficiently high potency, will, when prepared and stored under suitable conditions, retain for a considerable time a high degree of potency, and this notwithstanding that the extraneous organisms had been rapidly eliminated from it in an early stage of its preparation.

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